

Unusual Conformational Changes in 6-Hydroxymethyl-7,8-dihydropterin Pyrophosphokinase Revealed by X-ray Crystallography and NMR

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Introduction: Folates are essential for life. Unlike mammals, most microorganisms must synthesize folates *de novo*. 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) catalyzes pyrophosphoryl transfer from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP), the first reaction in folate pathway, and therefore, is an ideal target for developing novel antimicrobial agents. HPPK from *Escherichia coli* contains 158 amino acid residues and is thermostable, and thus, is a convenient model system for mechanistic studies. Crystal structures have been reported for HPPK without bound ligand (PDB entry 1HKA, 1998), containing an HP analog (1CBK, 1999), and complexed with an HP analog, two Mg^{2+} ions, and ATP (1DY3, 2000).

Methods and Materials: Single crystal X-ray diffraction and NMR.

Results: The crystal structure of *E. coli* HPPK in complex with MgADP has been determined at 1.5 Å resolution with a crystallographic *R*-factor of 0.191. The structure of the complex contains 153 of 158 amino acid residues of the polypeptide chain (residues 44-48 are disordered), one Mg^{2+} ion, one ADP molecule, one phosphate ion and 258 water molecules. The solution structure of HPPK in complex with MgAMPPCP has been determined using a simulated annealing protocol with 3,523 experimental NMR restraints. The root-mean-square deviation (RMSD) of the ensemble of 20 refined conformers that represent the solution structure from the mean coordinate set derived from them is 0.74 ± 0.26 Å for the backbone atoms of all residues. The RMSD is 0.49 ± 0.22 Å for the backbone atoms when residues P14, P44-Q50 and R84-P91 are excluded.

Conclusions: The bound MgADP in the crystal structure of HPPK•MgADP has two conformations. Binding of MgADP causes significant changes in the conformation and dynamical property of three loops of HPPK that are involved in catalysis. In comparison with the crystal structure of apo-HPPK, a dramatic, unusual movement of loop 3 has been revealed. Rather than moving in to close the active center of the enzyme as usually observed in substrate-induced fit, loop 3 moves away from the active center with some residues moving by >17 Å. The binding of MgADP also stabilizes loop 1 and loop 3 but makes loop 2 more mobile. Very similar conformational and dynamical changes are observed in the NMR solution structure of HPPK•MgAMPPCP. The conformational and dynamical changes may play important roles in facilitating both substrate binding and product release during the catalytic cycle.

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